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COMPARATIVE ANALYSIS OF PHENOLIC COMPOUNDS IN THE AMERICAN BASIL AND WILD BERGAMOT HERBS

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Abstract

The aim of this study was the comparative analysis of phenolic compounds in the *Ocimum americanum* and *Monarda fistulosa* herbs using high-performance liquid chromatography (HPLC) method and the spectrophotometric evaluation of the total hydroxycinnamic derivatives (THD) contents. Rosmarinic acid (RAc) was the predominant phenolic compound of both investigated species. RAc (22.84±0.61 mg/g) commonly with luteolin-7-*O*-glucoside, acacetin-7-*O*-glucoside, caffeic acid and chlorogenic acid can be considered as the quality markers of the *Monarda fistulosa* herb. The complex of polyphenols consisting of RAc (22.33±0.58 mg/g) followed by luteolin-7-*O*-glucoside, rutin and ferulic acid was specific to the *Ocimum americanum* herb. The THD content in the *Monarda fistulosa* herb (3.63±0.05 %) was higher comparatively to *Ocimum americanum* raw material (2.96±0.05 %).

Keywords: *Monarda fistulosa*, *Ocimum americanum*, *Lamiaceae*, *polyphenols*, *HPLC*, *spectrophotometry*

Introduction

Biologically active compounds isolated from various natural sources like plants, fungi, microorganisms and animals have long been used for the discovery of new drugs [1]. Many of natural constituents are almost impossible to imitate which makes them indispensable in treatment of human diseases [2–4].

In recent decades, there has been observed a narrowing of the distribution areas of a number of wild species of official medicinal plants on the European continent. It leads to the depletion of their raw materials sources and reflects the necessity of finding the new promising species of plants which accumulate the targeted bioactive compounds.

American basil (*Ocimum americanum* L.) and Wild bergamot (*Monarda fistulosa* L.) are the essential oil-bearing plants belonging to the *Nepetoideae* Burnett. subfamily of the *Lamiaceae* Martinov family. *Ocimum americanum* is native to Africa where used locally as a sedative and digestive remedy [4–6]. *Monarda fistulosa* is originated from North America where it was used by Indians for treatment colds, flu, headache and skin wounds. Thus, these species are succesfully used in folk medicine of different countries. Nowadays, both species are naturalized in Europe as easily cultivated ornamental and spicy species [5, 7, 8]. Generally, it opens the prospect of creating their proper raw materials base for using in the pharmaceutical industry.

The aerial parts of abovementioned species accumulate mainly such secondary metabolites as volatile terpenoids and polyphenols [6, 9–13] possessing a lot of valuable therapeutic properties [14]. Essential oil of these plants are the natural sources of constituents with antioxidant, antibacterial, antifungal, sedative, etc. actions [11, 12]. Phenolic compounds are regarded as very promising antioxidants synthesized in many *Nepetoideae* representatives [15–19]. The healing properties of polyphenols are mostly due to their free radical scavenging potential [20, 21].

Despite the noticeable interest of scientists in the phytochemical analysis of these species, their raw materials are not represented in any world Pharmacopoeias [22, 23]. Thus, they belong to the non-official medicinal plants. It indicates the inadequacy of their scientific research and restricts use in medical practice.

The aim of this study was the comparative analysis of phenolic compounds in the *Ocimum americanum* and *Monarda fistulosa* herbs using high-performance liquid chromatography (HPLC) method and the evaluation of the total hydroxycinnamic derivatives (THD) contents using differential spectrophotometry.

Methods

Plant material

The studied herbs were collected from the experimental plots in Ternopil region (Ukraine) during the flowering stage. Plants were growing from the seeds obtained from the new cultures department of M. Gryshko National Botanical Garden (National Academy of Sciences, Ukraine).

The herbs were dried in a shade at 25–35°C. The extractions of plant raw materials using water-ethanol solutions were carried out in a boiling water bath.

Chromatographic analysis

Chromatograph Shimadzu HPLC-DAD system equipped with the Phenomenex Luna C18 column (250 mm x 4.6 mm) was used for the HPLC analysis of polyphenols in the 70% ethanol extracts of herbs. The gradient elution was provided by mixing the mobile phases I (0.1% trifluoroacetic acid in water) and II (0.1% trifluoroacetic acid in acetonitrile). The injection volume was 5 μL and the flow rate was 1.0 mL/min [7]. The column temperature was 35°C. HPLC-grade acetonitrile and trifluoracetic acid were purchased from Sigma-Aldrich (USA). Reference standards of polyphenols were from Merck (Germany). The UV absorption spectra of the test samples and reference standards were recorded in the range of λ=190–400 nm.

Spectrophotometric analysis

The amount of THD was evaluated by the differential spectrophotometry method using spectrophotometer Lambda 25 UV/VIS (PerkinElmer). The analysis was conducted according to the Monograph 'Rosemary leaf' of the European Pharmacopoeia [23] and calculated as rosmarinic acid (RAc) equivalent. The light absorbance was measured at 505±2 nm. All the used chemicals were of analytical grade.

Statistical Analysis

Statistical analyzes were performed using the Statistica software (StatSoft, version 13.1).

Results and Discussion

The HPLC method was used for the quantification of phenolic compounds in the herbs of investigated species. The amounts of hydroxycinnamic acids (RAc, caffeic, ferulic, neochlorogenic, chlorogenic) and flavonoids (apigenin, luteolin, rutin, quercetin, catechin, hyperoside, acacetin-7-*O*-glucoside, apigenin-7-*O*-glucoside, luteolin-7-*O*-glucoside) were evaluated (Table 1, Figures 1, 2).

The most of found hydroxycinnamic acids, flavonols and flavones possess the proven therapeutic activities [24–27]. Some of them could be considered as the quality markers of the studied plant raw materials [28].

RAc was the major phenolic compound of both investigated species as well as many other *Nepetoideae* representatives [16, 29–31]. RAc demonstrates noticeable antioxidant, anti-inflammatory, immunomodulatory, etc. properties [32–34]. RAc isolated from the *Salvia verticillata* shown the anti-SARS-CoV-2 properties [35]. Shanaida et al. [31] revealed that RAc was the predominant polyphenol of the methanol extracts of *Monarda fistulosa* (20.32 mg/g) and *Ocimum americanum* (19.59 mg/g) herbs studied by high-performance thin layer chromatography method. 7.84 mg/g of RAc was detected by Jakovljević et al. [36] in the *Satureja montana* herb. Leaves of *Salvia verticillata* leaves accumulate 12.31 mg/g of this hydroxycinnamic acid [37]. Benedec et al. [16] investigated several *Nepetoideae* representatives. The *Origanum vulgare* aerial part had the maximum content of RAc (12.40 mg/g) among them [16].

As can be seen from Table 1, the contents of caffeic and chlorogenic hydroxycinnamic acids were higher in the *Monarda fistulosa* herb comparatively to *Ocimum americanum* (3.98 and 14.59 times, respectively). The content of ferulic acid was 4.14 times higher in the herb of *Ocimum americanum*. Toma et al. [38] and Khan et al. [39] revealed the free radical scavenging effects of caffeic acid. According to Miao and Xiang [40], chlorogenic acid is characterized by the antioxidant, anticancer, immunomodulatory, and anti-inflammatory properties. Michels et al. [41] found that ferulic acid and its derivatives can cause the memory enhancement.

Luteolin-7-*O*-glucoside was the predominant flavonoid of both studied species. The content of this flavone was 3.92 times higher in the *Monarda fistulosa* herb when compared to *Ocimum americanum*. The amount of flavonol rutin was much higher in the *Ocimum americanum* herb (2.94 mg/g) comparatively to *Monarda fistulosa* (0.71 mg/g).

The antioxidant and anti-inflammatory effects of the revealed flavonoids were proven by researchers [42, 43]. Rutin can deliver the more biologically active aglycon quercetin in the digestive tract which can significantly suppress the inflammatory process [42]. The established dominance of flavonoid glycosides over aglycones can be considered an indicator of the proper quality of harvested raw materials. This is consistent with the statement that as the organs of plants age, they accumulate mainly aglycones of flavonoids [31].

As the antioxidant effect depends on the number and location of hydroxyl groups, the major polyphenolic components of investigated herbs such as rosmarinic acid or rutin with 4 OH-groups and luteolin-7-*O*-glucoside containing 3 of them possess the significant free radical scavenging activity [16, 19].

It could be supposed that polyphenols commonly with terpenoids which are extracted from the *Nepetoideae* plants using water-ethanol solutions possess the prominent pharmacological properties and may act synergistically [44, 45].

The evaluation of THD contents in the investigated herbs was implemented using the differential spectrophotometry. It was conducted the comparative analysis of electronic absorption spectra of the obtained 50% ethanol extracts of herbs and the RAc standard (before and after the formation of their complexes with nitrite-molybdenum reagent). It was detected the coincidence of the light absorption maxima of the studied extracts and standard of RAc at a wavelength of 505±2 nm. Thus, the quantifications of the THD amounts were determined in terms of RAc equivalent.

As can be seen from the obtained data (Figure 3), the content of THD in the *Monarda fistulosa* herb

(3.63±0.05 %) was higher when compared to *Ocimum americanum* (2.96±0.05 %) raw material.

The obtained results on the THD contents in the *Monarda fistulosa* and *Ocimum americanum* herbs are close to the results of the study of the other species belonging to the *Nepetodeae* subfamily [25, 46]. Thus, among 32 Croatian *Lamiaceae* species, the members of the genus *Melissa, Origanum* and *Satureja* were found to be the richest in the THD [46].

Conclusions

As shown by the results of HPLC analysis, RAc commonly with luteolin-7-*O*-glucoside, acacetin-7-*O*-glucoside, caffeic acid and chlorogenic acid can be considered as the quality markers of the *Monarda fistulosa* herb. The complex of polyphenols consisting of RAc followed by luteolin-7-*O*-glucoside, rutin and ferulic acid was specific to the *Ocimum americanum* herb. It should be noted that the revealed predominant polyphenolic molecules possess the proven therapeutic properties. The THD contents measured spectrophotometrically was 1.23 times higher in the *Monarda fistulosa* herb comparatively to *Ocimum americanum*.

The conducted studies revealed that *Monarda fistulosa* and *Ocimum americanum* herbs could be regarded as the perspective sources of polyphenols with significant pharmacological potential.

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Table 1. Contents of phenolic compounds in the investigated species evaluated by HPLC

Compound	Retention time, min	Content, mg/g	
		Ocimum americanum	*Monarda fistulosa*
Neochlorogenic acid	14.8	0.08±0.01	1.63±0.07
Catechin	19.5	0.06±0.01	0.09±0.01
Chlorogenic acid	20.4	0.22±0.01	3.21±0.09
Caffeic acid	21.6	1.24±0.03	4.93±0.15
Rutin	30.9	2.94±0.08	0.71±0.02
Hyperoside	31.6	1.82±0.05	0.53±0.02
Ferulic acid	32.3	2.82±0.06	0.11±0.01
Luteolin-7-*O*-glucoside	33.1	4.83±0.13	18.92±0.65
Apigenin-7-*O*-glucoside	36.8	1.31±0.04	1.84±0.05
RAc	37.8	22.33±0.58	22.84±0.61
Acacetin-7-*O*-glucoside	45.8	0.92±0.03	3.11±0.08
Quercetin	46.6	0.80±0.03	0.07±0.01
Luteolin	47.0	2.23±0.06	2.78±0.07
Apigenin	52.4	0.64±0.02	1.41±0.05

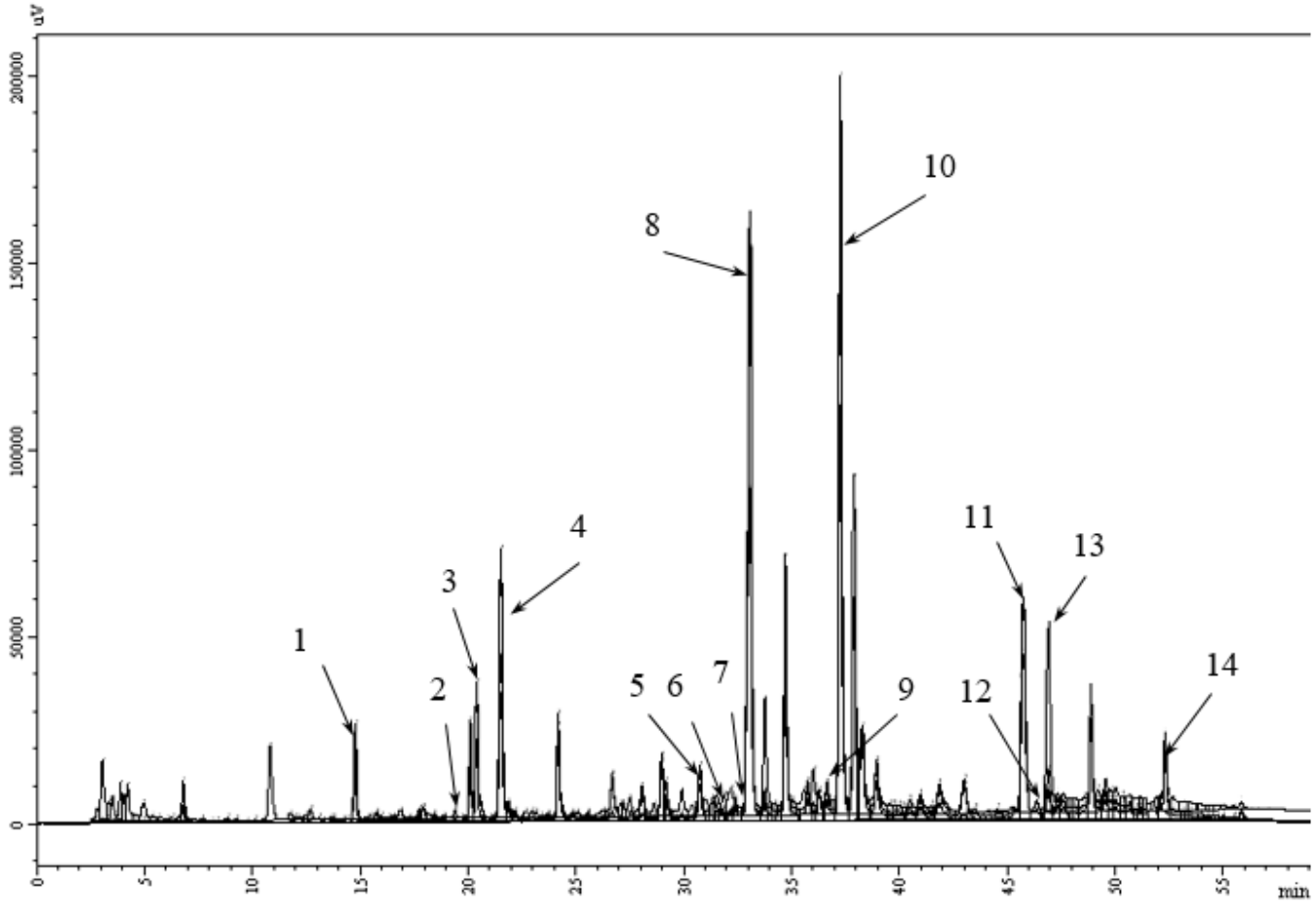

Figure 1. Typical HPLC chromatogram of polyphenols in the *Monarda fistulosa* herb: 1 – neochlorogenic acid; 2 – catechin; 3 – chlorogenic acid; 4 – caffeic acid; 5 – rutin; 6 – hyperoside; 7 – ferulic acid; 8 – luteolin-7-*O*-glucoside; 9 – apigenin-7-*O*-glucoside; 10 – RAc; 11 – acacetin-7-*O*-glucoside; 12 – quercetin; 13 – luteolin; 14 – apigenin.

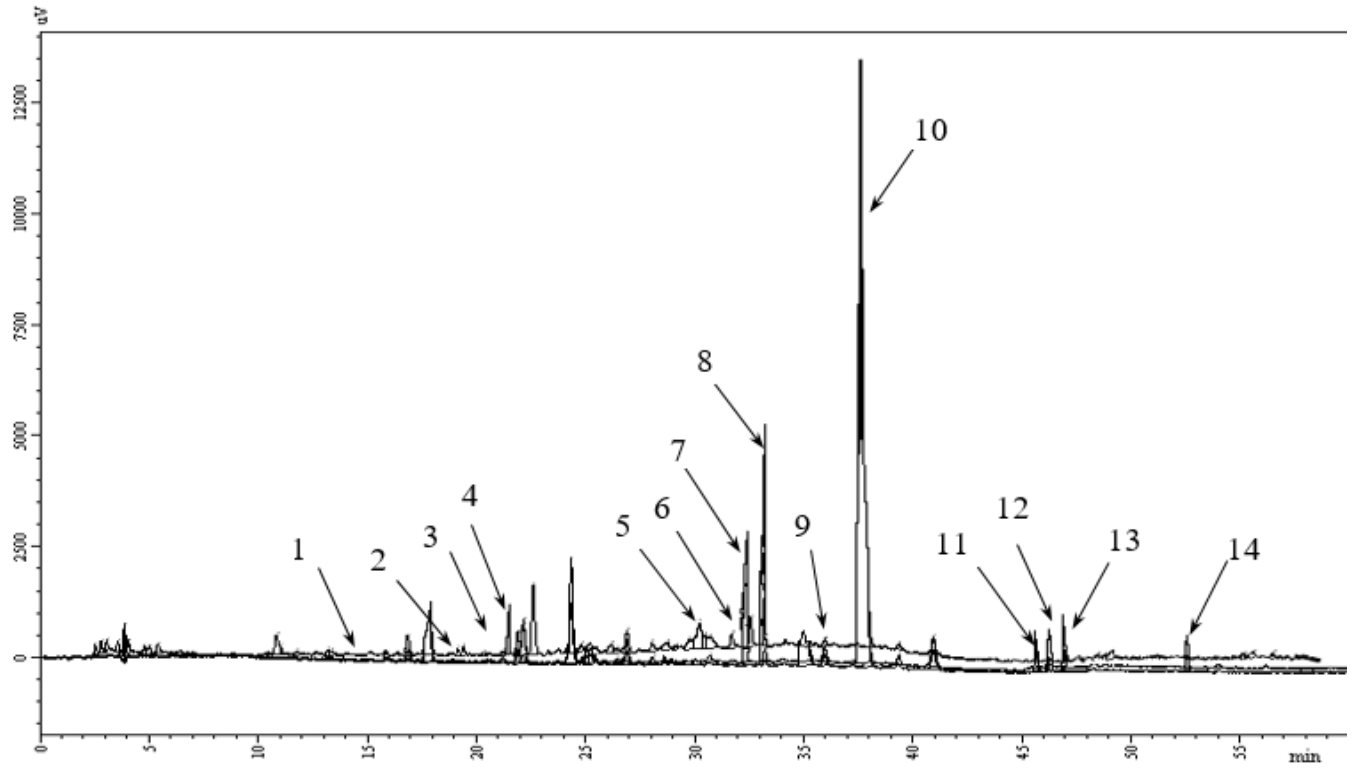

Figure 2. Typical HPLC chromatogram of polyphenols in the *Ocimum americanum* herb: 1 – neochlorogenic acid; 2 – catechin; 3 – chlorogenic acid; 4 – caffeic acid; 5 – rutin; 6 – hyperoside; 7 – ferulic acid; 8 – luteolin-7-*O*-glucoside; 9 – apigenin-7-*O*-glucoside; 10 – RAc; 11 – acacetin-7-*O*-glucoside; 12 – quercetin; 13 – luteolin; 14 – apigenin.

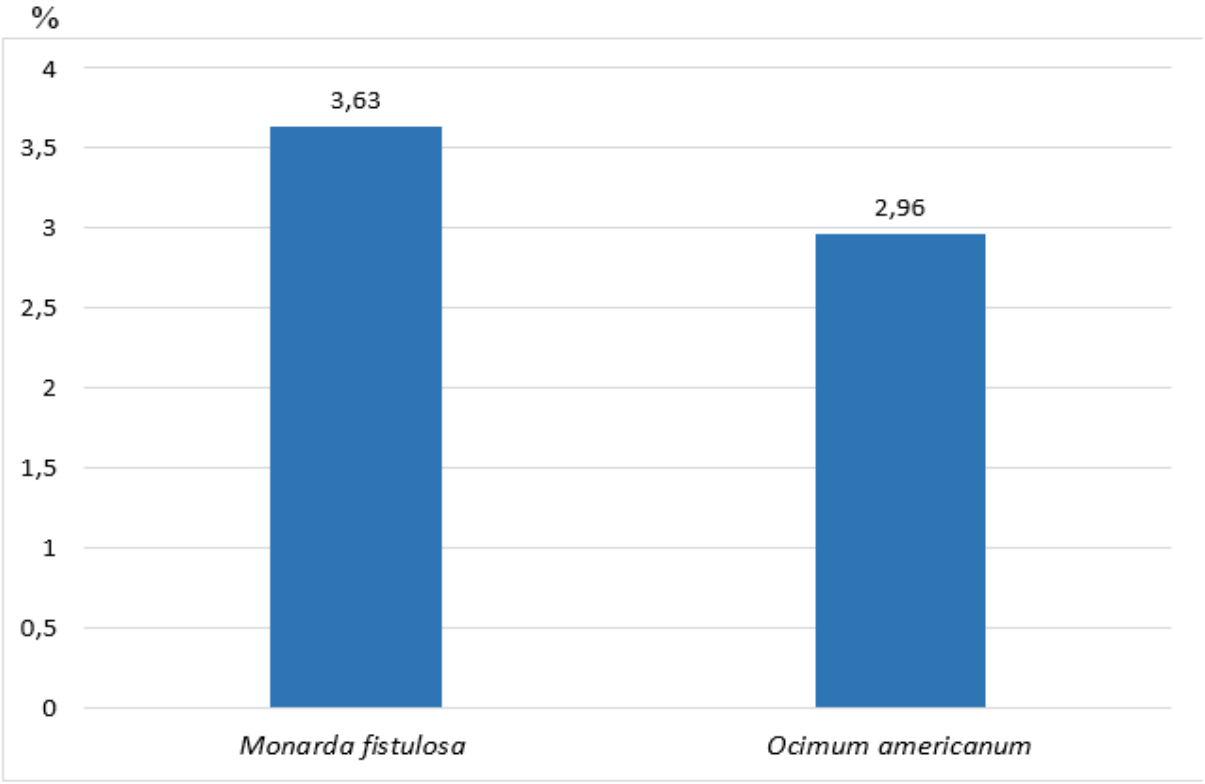

Figure 3. Contents of the THD in the herbs of investigated species